APR 30 1951
B 298979

VOLUME 9 NUMBER 4

NUTRITION REVIEWS

April 1951

THE LIBRARY OF CONGRESS SERIAL RECORD
MAY - 2 1951
COPY

Industrial Nutrition 97
Estrogen and Calcium Metabolism 100
Prevention of Tooth Decay by Tube Feeding 101
Liver Protein and Enzyme Activity 103
Potassium-Phosphorus Interrelationship 104
Protection Against Radiation Damage With Cysteine 106
Therapy of Polycythemia 108
Utilization of Nonprotein Nitrogen 109
Metabolism of Phytin Labeled with P^{32} 111
Glutamic Acid and Mental Functions 113
Effect of Pancreatectomy on Synthesis of Plasma Phospholipid 117
Relation between Tocopherol and Carotene Content of Milk and Its Resistance to Oxidized Flavors 118
Influence of Folacin and Vitamin B_{12} on Pyridine Toxicity in Rats 119
Epithelial Pigmentation and Copper Deficiency 121
Vitamin E in the Nutrition of Swine 122
Reticulocyte Ripening and Amino Acids 124
Wernicke's Syndrome 126
Notes
Letter to the Editor 127
Armed Forces Rations 127
Clinical Nutrition Briefs 128

Published Monthly by
THE NUTRITION FOUNDATION, INC.
CHRYSLER BUILDING NEW YORK 17, N. Y.

COPYRIGHT, 1951, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

CHRISTINE S. WILSON, 695 Huntington Ave., Boston

Associate Editors

JOSEF BROŽEK, PH.D., University of Minnesota, Stadium South Tower, Minneapolis
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
R. W. ENGEL, PH.D., Alabama Polytechnic Institute, Department of Animal Husbandry and Nutrition, Auburn
JEAN MAYER, PH.D., D.Sc., Department of Nutrition, Harvard School of Public Health, Boston
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ARTHUR H. SMITH, PH.D., Wayne University College of Medicine, Detroit
ESMOND E. SNELL, PH.D., Department of Biochemistry, University of Wisconsin, Madison
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
J. MURRAY LUCK, PH.D., Stanford University
JAMES S. McLESTER, M.D., University of Alabama
HENRY C. SHERMAN, PH.D., Columbia University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
RUSSELL M. WILDER, M.D., University of Minnesota
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

APR 30 195
B 298979

NUTRITION REVIEWS

VOL. 9 APRIL, 1951 No. 4

INDUSTRIAL NUTRITION

Interest in the relationship between the nutrition of industrial workers and their health and efficiency has greatly increased during recent years. In 1924, when unemployment was widespread, the British Medical Research Council (*Special Report Series* **87,** (*1924*)) investigated the adequacy of the diet of coal miners and their families; and in 1936 the International Labor Office (League of Nations) issued a report (*Studies and Report Series B* (*Social and Economic Conditions*) **23,** (*1936*)) on workers' nutrition and social policy. Since that time many other studies have been made, notably those under the auspices of the Milbank Memorial Fund.

The practical importance of the application of nutritional principles to the conditions existing in industry was greatly increased during the years of World War II. In Britain during the most critical years of industrial mobilization in November 1940, the Factories (Canteens) Order was passed directing any factory employing more than 250 people to make satisfactory arrangements for establishment and maintenance of a suitable canteen where hot meals could be purchased by the factory workers. The Order was at first applied only to plants engaged on munitions work, but in April 1943, when its value had become apparent, it was extended to cover all factories doing work considered to be in the national interest.

Those responsible for the well-being of industrial workers quickly appreciated that provision of adequate meals designed to ensure that the total daily diet of each worker was nutritionally adequate for his or her needs was not only an important step forward in social practice but was also calculated to increase industrial efficiency. Now that once again the industrial effort of the western world is being intensified, it is pertinent to review the principles by which the nutritional health of factory workers may be achieved.

Human nutrition may be considered a branch of applied physiology. As such it is a science that takes no account of the subjective feelings of the people being fed. The nutritional well-being of the animal species *Homo sapiens* is as little affected by his likes and dislikes as are the nutritional requirements of experimental rats confined in a laboratory. From the rigid standpoint of animal physiology, the average nutritional requirements of groups of people are fixed and are based on such measurable characteristics as sex, age, height, weight, degree of activity, rate of growth, and sexual maturity. Furthermore, when average groups are considered, there are no exceptions to the laws of physiology. The nutritional needs of similar individuals are the same whatever type of diet they may happen to be accustomed to eat.

This raises an important point for the industrial nutritionist. The nutritional requirements of similar individuals are the same but their customary diets may be very different. The variety of diets eaten by different groups within a country may be large. Many of these groups subsist on inadequate diets and suffer in consequence, but it may happen that diverse diets equally well provide the nutritional needs of those eating them. No matter how unusual a traditional diet may appear, it ought never to be condemned until its nutritional composition is known. The industrial nutritionist needs to conduct himself with humility. Bizarre customs among men working at unusual occupations have before now been

found well suited to the conditions under which they were followed.

The most direct way in which industrial work affects the nutritional requirements of the worker is through its calls on the body for physical energy, and hence its demand on the diet for calories. This is an important matter which has not been adequately studied under practical conditions. Although the scientific literature contains a considerable amount of information about the energy requirements of work, much of it is academic and of limited value to those who have the practical task of feeding actual workers.

Industrialists have for many years been aware that it is wasteful to use human muscle-power as a prime mover in a factory. As long ago as 1935, Hanssen at a meeting of the Institute of Civil Engineers in London, in discussing Moss's work on the calorie requirements of coal miners (*K. N. Moss, J. Inst. Civil Engineers* **1,** *354 (1935–36)*) pointed out that when a man exerted a manual output of one-eighth of a horsepower (h.p.), for which he received then the wage of 1s. 3d. an hour, 1 h.p. cost at least 10s. Since the mechanical efficiency of a man falls if he is compelled to work in a stooping posture, the cost of 1 h.p. could rise to 28s. 9d. if the miner was working in constrained conditions, whereas the same power could be obtained by mechanical means for a penny. The modern trend has, therefore, been to lighten the physical demands on the workman by increasing the mechanical power at his disposal. Up-to-date knowledge of the precise nature of the work carried out in a factory is, consequently, essential for intelligent designing of industrial meals. For example, in one plant "heavy press operation" may be a man's job calling for strenuous physical work, whereas elsewhere a task of the same name may be done almost entirely with automatic mechanical equipment, and perhaps by a female operative.

The basic requirement of a good diet is that it should provide the amounts of nutrients required for optimum health by the people eating it. These nutrients must, however, be supplied as food and the food must be provided as meals. The appropriate number of meals for an individual doing a particular job is a matter of considerable nutritional interest. It is also a subject upon which finality has not been reached. Part of the uncertainty arises from confused thinking and a lack of accurate observation. For example, when early plans for industrial feeding were being drawn up in Britain, it was assumed in some quarters that workpeople ate three meals a day. This, however, was soon found rarely to be so. If a meal is defined in nutritional terms it has to be described in some such words as "a meal is any occasion during which food providing 200 calories is consumed at one sitting." When this type of definition was applied it was found that some working people ate four meals a day, most of them ate five, and many, who had breakfast, a midshift snack, dinner, another snack, tea (the meal), and supper, ate six meals a day (M. Pyke, "Industrial Nutrition," *Macdonald & Evans, London (1950)*). The choice of a dietary pattern appropriate to the type of job a worker is doing always affects his or her contentment and well-being. It may also, under certain circumstances, be important for maintenance of health, and will certainly affect the efficiency with which work is done.

Besides the frequency of an industrial worker's daily meals, timing of them is also a matter of nutritional significance. Shift work affects the conventional pattern of the daily diet. Even a slight change in timing of the working hours may entirely change the type of meal best suited to the men or women concerned and will in consequence change the type of meal the works canteen should supply if it is properly to fulfill its function. The most extreme example of the influence of shift times on dietary needs is night-shift working. Night work raises a

number of physiologic and nutritional problems which it is impossible to discuss in a short space.

Investigations of problems of industrial nutrition carried out in recent years have served to demonstrate the complexity of the subject and the need for any approach to be conceived on a broad basis. A substantial body of experienced industrial opinion favors provision of frequent meals. This is supported by the physiologists on the basis that an adequate blood glucose level is needed for maximum muscular efficiency. A. Keys (*Fed. Proc.* **2,** *164* (*1943*)), however, has pointed out that the ideal dietary for people who do muscular work cannot be determined easily by laboratory experimentation. Above all, the classic Hawthorn Experiment of F. J. Roethlisberger and W. J. Dickson ("Management and the Worker," *Harvard Univ. Press* (*1941*)) shows clearly that industrial efficiency may be affected by a wide variety of factors partly psychologic, partly physiologic, and partly social among which meal-frequency may be a significant factor.

Industrial nutrition must deal for the most part with large groups of individuals and must, in consequence, make provision for their average nutritional requirements. But within each category it is now known that there exist individuals with needs differing materially from the average. Apart from differences of age, height, weight, activity, and so forth, for which provision can be calculated according to the principles set out, for example, in the recommended allowance of the Canadian Council of Nutrition (*Canad. Bull. Nutrition* **2** (*No. 1*) (*1950*)) there are substantial differences in nutritional demands which can at present be attributed only to individual idiosyncracy. These have been pointed out by many students of nutrition including E. M. Widdowson (*J. Hyg.* **36,** *269* (*1936*)) and more recently C. Delbue, R. Passmore, J. Thomson, and J. A. Watt (*Brit. J. Social Med.* **3,** *139* (*1949*)). A further aspect of the differences which may occur in the physiologic demands of individuals is the existence of tall thin people, short fat people, square hairy people, and all the diverse varieties of human physique which W. H. Sheldon ("The Varieties of Human Physique," *Harper, New York* (*1940*)) has shown to differ so markedly from each other. The potential importance of these considerations to the industrial nutritionist is immediately apparent.

Industrial nutrition is more than a branch of applied biochemistry, it is a social science. Those who are responsible for industrial health must, therefore, know more than the principles of nutrition. Health has recently been defined by the World Health Organization at their meeting in May 1950 as "a state of complete physical, mental and social well-being." Modern industrial work can affect those employed in it in all three respects. In Britain during World War II, industrial canteens were established to maintain the physical well-being of the workmen, but equally to support their social well-being, since their work took them away from their homes at meal times, and for many of them took their wives away to work as well.

The person in charge of a works canteen must complete a diet, part of which is eaten by the workers at home. The nutritionist must, therefore, study the conditions of home life as well as working conditions in the factory. Even when the canteen meals have been designed to supply the nutrients required to complete a nutritionally satisfactory diet they will fail in their purpose if those for whom they are designed refuse to eat them. The industrial nutritionist must first of all apply the discipline of nutritional science to the problem at hand, but if he is to succeed fully he must take a wider sweep and remember that he is dealing with

human kind—people whose lives, for good or evil, are passing away; people who are influenced by hopes and fears and likes and dislikes, economic motives, altruistic motives, or common mischievousness. In fact, industrial nutrition is a part of industrial health and industrial health deals with the whole man and the whole woman whose paths bring them into industry.

MAGNUS PYKE
Yeast Research Outstation,
The Distillers Company Ltd.
Glenochil, Clackmannanshire
Scotland

ESTROGEN AND CALCIUM METABOLISM

While the role of the parathyroid hormone in regulating blood calcium levels has been known for a relatively long time, the possible role of other hormonal factors in calcium metabolism has remained largely unknown. A publication by M. J. Dallemagne, J. Govaerts, and J. Mélon (*Arch. internat. de physiol.* **58,** *157* (*1950*)) dealing with the influence of estrone on the calcium metabolism of the pigeon indicates that female sex hormones have a determining effect.

P. Kyes and T. S. Potter (*Anat. Record* **60,** *377* (*1934*)) had shown that during the period of sexual activity the cavity of the bones of the female pigeon "fills up" with bone while that of the male remains filled with marrow. This observation was later extended to numerous avian species and W. Landauer, C. A. Pfeiffer, W. U. Gardner, and E. B. Man (*Proc. Soc. Exp. Biol. Med.* **41,** *80* (*1939*)) showed that this new growth of bone could be duplicated by injecting estrogens.

J. Benoit, G. Fabiani, R. Grangaud, and J. Clavert (*Bull. Soc. chim. biol.* **23,** *1981* (*1941*)) found that administration of estrogens was accompanied by an increase in the blood calcium level. During the period of sexual activity in the female, or during the period of estrogen administration, the animal is in positive calcium balance and intestinal absorption of calcium increases. This is reversed when the period of sexual activity is over or when estrogen administration is discontinued (Clavert and Benoit, *Ibid.* **24,** *1469* (*1942*); Clavert, *Arch. d'anat. micr.* **37,** *41* (*1948*)). Analogous effects on phosphorus metabolism have been demonstrated (J. Govaerts and M. J. Dallemagne, *Nature* **161,** *977* (*1948*)).

Dallemagne, Govaerts, and Mélon (*loc. cit.*) studied the influence of estrone on calcium metabolism using Ca^{45} as a tracer. Five groups of animals were used, all receiving injections of radiocalcium: control males, males receiving estrogen during short periods, males receiving it during long periods, female animals in a state of spontaneous sexual activity not receiving estrogen, and female animals in this state receiving estrogen. At the conclusion of the experiment (after thirty-three days) the animals were killed by bleeding; chemical analyses and determinations of the radioactive content were performed on the skeleton. Chemical determinations and radioactive counts were also performed on the other tissues, blood, liver, intestine, and skeletal muscles in particular.

The effects of estrogen administration were striking, both on the skeleton and the soft tissues. Considerable increases of absorption and retention of the dietary calcium were noted. These effects became more marked as administration of estrogen was continued, maximum effects being obtained after seven days. Intestinal resorption of calcium began to decrease after seventeen days. Not only was new bone laid down to replace bone marrow but the rate of turnover of the calcium of old bone tissues was increased, particularly in the diaphyses. This effect was found not to be quantitatively the same for all bones, femurs and tibias being particularly affected, humeri being relatively inac-

tive. While the "biological life expectancy" of calcium atoms was increased under the influence of estrogen administrations from forty-five to seventy-five days, the time necessary for equalization of the specific calcium radioactivity of blood and femur or tibia was definitely shortened from more than twenty-three days to from nine to sixteen days.

Retention of calcium under the influence of circulating estrogen in the female bird appears correlated with accumulation of calcareous material necessary for formation of the shells of eggs. It appears likely that in mammalians the ever increasing concentration of estrogen (theelin-theelol) in blood from the beginning to the end of pregnancy corresponds to the ever increasing calcium requirements of the developing fetal skeleton.

In addition to the evident importance of the hormones in developmental biochemistry, the effect of estrogens on calcium metabolism may explain the wide range of variation in calcium nutrition found between individuals on widely different calcium intakes. It may also be more easily understandable that low calcium intakes often do not result in as serious consequences as could be expected if in conditions of higher requirements, like puberty or pregnancy, a physiologic adaptation based on endocrine changes takes place which increases calcium absorption as well as calcium retention.

PREVENTION OF TOOTH DECAY BY TUBE FEEDING

Accumulation of food debris in the mouth has long been suspected as one of the causes of tooth decay. Workers at the Harvard School of Dental Medicine have made the interesting observation that tooth decay can be prevented in rats by feeding them with a stomach tube so that food does not come in contact with the teeth (O. W. Kite, J. H. Shaw, and R. F. Sognnaes, *J. Nutrition* **42,** *89 (1950)*).

The animals used in this investigation were offspring of rats that had been on tooth decay-producing diets for two previous generations. Under ordinary circumstances a high incidence of tooth decay would be expected in such animals.

The diet consisted of the following (percentage): powdered sugar 67; blood fibrin 24; corn oil 5; and salt mixture 4. It was supplemented with 2 per cent each of whole liver substance and 1:20 liver concentrate powder; the following vitamins (milligrams per 100 g. of diet): thiamine hydrochloride 0.35, riboflavin 0.35, pyridoxine hydrochloride 0.35, calcium pantothenate 2, niacin 2.5, choline chloride 100, inositol 100, para-aminobenzoic acid 30, beta-carotene 1.1, alpha-tocopherol 5, menadione 0.6; and with 300 I.U. of irradiated ergosterol per 100 g. of diet.

Control rats were allowed to eat the diet and drink water ad libitum. The rats that were fed by stomach tube received the diet in the form of a water suspension, which contained 3.3 to 3.7 calories per milliliter (two parts of diet to one part of water). Some animals that had their salivary glands removed were also included in the study.

Ninety-two rats were used in the study, 65 intact and 27 desalivated. The animals initially weighed from 27 to 86 g. and were placed on experiment at weaning or shortly thereafter. The experimental period varied from sixteen to twenty-five weeks.

In the early days of the experimental period, when the rats were small, considerable difficulty was encountered with the mechanics of tube feeding, and consequently survival was rather poor. The deaths that occurred were the result of accidental passage of the tube into the trachea, puncture of the esophagus or stomach, or overloading the stomach. Only 16 of the 52 intact tube-fed rats and only 3 of the 23 desalivated

tube-fed rats survived the experimental period. Thirty-two of the 56 deaths occurred during the first five days of the study. No deaths occurred in the 13 intact and the 4 desalivated rats that were allowed to eat the diet ad libitum.

All of the tube-fed rats that survived the experimental period remained free of tooth decay. In contrast, the 13 intact control rats had an average of 5 decayed molars and an average of 6.7 carious lesions and the 4 desalivated controls had an average of 11.5 decayed molars and an average of 28.8 carious lesions. The differences in incidences of decayed molar teeth and carious lesions between those animals fed normally and those fed by way of stomach tube were highly significant.

The complete prevention of tooth decay in the rats fed by way of stomach tube is particularly significant because, pre-experimentally, the rats had been conditioned by a dietary regimen which made them generally susceptible to tooth decay. These results thus establish that food had to be present in the oral cavity in order for carious lesions to develop in susceptible animals.

The average growth rate of the rats receiving the diet by way of stomach tube was superior to that of the rats eating the same diet ad libitum. The authors point out that reduction in tooth decay in tube-fed animals could thus not be attributed to caloric restriction, a condition which is known to reduce dental decay significantly (J. H. Shaw, *J. Am. Dietet. Assn.* **24,** *181 (1948)*).

The oral cavities of the rats were not subjected to chemical or bacteriologic tests. Microorganisms, however, were demonstrated histologically to be present round the teeth of both control rats and those fed by way of stomach tube. In the tube-fed rats a heavy, transparent membrane, presumably the Nasmyth's membrane or the "skin of the teeth," seemed to be present routinely between the enamel surface of the teeth and the microorganisms. In the control rats there was no evidence of this membrane.

Under normal eating habits Nasmyth's membrane is generally considered to be destroyed soon after tooth eruption. The Harvard investigators noted that the teeth of the tube-fed animals could be distinguished from the controls not only by the absence of caries but also by the lack of stainability of the enamel and by lack of a direct contact of enamel with microorganisms. No differences were noted in the tooth-supporting structures (bone, periodontal membranes, and cementum) of tube-fed and control rats. The latter observation is of interest in view of the greatly reduced masticatory function among the tube-fed rats.

Prevention of tooth decay by tube feeding in desalivated rats is particularly noteworthy because other investigators had shown earlier that removal of the salivary glands greatly increased the incidence of tooth decay in rats, and the results of the present study with desalivated control rats confirm this earlier observation.

Probably the most important observation reported in this paper is the difference in stainability of the enamel in the tube-fed and the control rats. The lack of penetration of stains into the enamel of the tube-fed rats is a fundamental characteristic of mature, fully calcified enamel. On the other hand, the relatively immature enamel of erupting teeth, or of recently erupted teeth, is penetrable by stains; likewise enamel which has been etched or partially decalcified by chemical treatment is readily penetrated by certain histologic dyes. Apparently the enamel of the teeth of the control rats had never attained a full maturity of calcification due to the presence of this particular diet in their environment, or this diet, combined with other factors in the environment, had caused a partial generalized decalcification of the enamel surface. Unquestionably the present paper raises

many questions on the maturation and maintenance of enamel and on the relation of enamel substance to caries resistance.

The technic of tube feeding will no doubt serve as a valuable approach to future studies on the initiation and development of tooth decay. It will obviously serve as a useful procedure for assuring an adequate intake of all essential nutrients and at the same time permit a study of the influence of individual nutrient factors in the oral environment on tooth decay.

LIVER PROTEIN AND ENZYME ACTIVITY

The question of whether functional demand conditions the rate of formation of enzymes has been argued for a long time. Pavlov believed that the character of foods in the gastrointestinal tract influenced the quantitative makeup of the digestive enzyme mixture which was secreted into the gut. Although it has proved difficult to secure definitive experimental data on this point, efforts have been made to elucidate the effect of other factors on enzyme production.

Inasmuch as enzymes are proteins it would seem that those influences which alter the protein content of organ tissues might be expected to change the production of enzymes by those tissues. The answer to this question can conceivably be approached through nutritional adjustment.

Some years ago T. Addis, L. J. Poo, and W. Lew (*J. Biol. Chem.* **115,** *111, 117 (1936)*; **116,** *343 (1936)*) showed that during inanition the liver loses its protein more rapidly than any other of the tissues studied. Hepatic tissue contains a large number of enzymes, several of which have actions which are readily demonstrated experimentally, and it would, therefore, seem promising to investigate the effect of the level of dietary protein upon the protein content of the liver and its concomitant production of enzymes.

The experimental device of inanition was used by L. L. Miller (*J. Biol. Chem.* **172,** *113 (1948)*) to deplete liver proteins. Adult albino rats of both sexes were given an adequate purified diet for ten days; then they were divided into two groups, one of which served as controls and continued to eat the ration, whereas the other was given water only. After seven days both groups were killed and a cell-free homogenate of the liver was prepared upon which the following determinations were made: total protein, catalase activity, alkaline phosphatase, xanthine dehydrogenase, and cathepsin. The activity of the enzymes was expressed both as "units per 100 g. of body weight" and as "units per gram of liver protein." After a fasting period of seven days the rat-liver catalase, alkaline phosphatase, xanthine dehydrogenase, and cathepsin decreased in liver tissue at about the same rate as did the liver protein. The author concluded that this loss of enzyme activity could not be attributed to a decrease in available prosthetic groups or enzyme activators or to an accumulation of enzyme inhibitors, but rather it probably represented a loss of enzyme protein itself.

In a later study (L. L. Miller, *J. Biol. Chem.* **186,** *253 (1950)*) the experiment was made more definitive by using rations with high and low protein contents; the control ration contained 25 per cent casein, the low protein diet contained 6 per cent casein, and a third ration contained no protein. Realimentation was carried out by using the control diet. In addition to the enzymes catalase, xanthine dehydrogenase, alkaline phosphatase, and cathepsin, liver arginase was studied at the various levels of dietary protein. Again it was found that in those animals maintained for twenty-one to twenty-three days on protein-poor or protein-free diets there is a loss of the various enzymes meas-

ured which is associated with a decrease in liver protein. When such animals were realimented, both the activity of the enzymes and the level of protein were restored.

That the observed response is closely related to the quality of the dietary protein was demonstrated by feeding one group of rats on the 6 per cent casein ration to which was added enough glycine to bring the level of total nitrogen to that of the control (25 per cent casein) ration. In these animals there occurred the same loss of enzyme activity as observed on the low protein or nonprotein diets indicating that protein of adequate biologic value must be available for synthesis of enzyme proteins as well as for synthesis of body tissue.

This general view is supported in other species and with other enzymes. Thus in fasted rabbits (J. Schultz, *J. Biol. Chem.* **178,** *451* (*1949*)) the total nitrogen showed a decrease as did the activity of cathepsin II, and the conclusion was drawn that the relatively labile protein of the tissue as contrasted to the stable cellular protein is effective in supporting enzyme activity. Again in a study of rats given a protein-free diet and pair-fed with control animals (S. Seifter, D. M. Harkness, L. Rubin, and E. Muntwyler, *Ibid.* **176,** *1371* (*1948*)), the loss in hepatic nitrogen was directly related to the decreased activities of D-amino acid oxidase and of arginase.

In other experiments (V. R. Potter and H. L. Klug, *Arch Biochem.* **12,** *241* (*1947*)) diets high in carbohydrate or rich in fat, but in each case low in protein, induced a diminished activity of succinoxidase and of fatty acid oxidase in the liver of rats. From the available evidence there is a strong suggestion that liver-enzyme proteins enter into the general protein metabolism of the body in a dynamic but more or less nonspecific way.

POTASSIUM-PHOSPHORUS INTERRELATIONSHIP

The importance of dietary phosphorus for normal bone calcification has been studied extensively in several species of animals, particularly in chicks. The importance of potassium in the calcification process was not appreciated until M. B. Gillis (*J. Nutrition* **36,** *351* (*1948*)) reported that a liberal potassium intake increased the bone ash values in chicks receiving diets low in phosphorus. This observation suggested a relationship between potassium and phosphorus metabolism. The same investigator has now reported more detailed studies with chicks and also with rats (*Ibid.* **42,** *45* (*1950*)).

In the first experiment with chicks graded quantities of potassium were added to each of two diets that contained 0.30 and 0.60 per cent of phosphorus, respectively. The calcium content of the two diets was the same, i.e., 0.75 per cent. In the second experiment with chicks graded quantities of potassium were added to each of two diets that contained 0.35 and 0.60 per cent of phosphorus, and 0.70 and 1.20 per cent of calcium, respectively. These diets thus permitted an evaluation of potassium influence with several different dietary calcium-phosphorus ratios.

The chick diets contained the following components: dried blood fibrin 20; gelatin 4; ground cellophane 3; soybean oil 3; fish liver oil 0.5; liver "L" 1; salt mixture 0.8; vitamin mixture 0.4; phosphorus, calcium, and potassium in the desired amounts; and corn starch to equal 100. The diets were analyzed for potassium by means of flame photometry. Dicalcium phosphate, calcium carbonate, and potassium chloride served as sources of the minerals under study.

These diets, when optimum with respect to phosphorus and potassium content, produced gains of approximately 250 g. per chick in the four-week experimental period.

No chicks survived the four-week experi-

mental period when the diet contained less than 0.12 per cent of potassium. Deficiency symptoms noted were retarded growth, weakness, loss of the use of the legs, enlarged kidneys, and excretion of large amounts of urates.

The diets containing 0.30 or 0.35 per cent of phosphorus and 0.24 per cent of potassium produced about 200 g. of gain in weight per chick in four weeks. With lower levels of dietary potassium there was a decline in body weight gains. The diets containing 0.60 per cent of phosphorus and 0.20 per cent of potassium produced somewhat better weight gains (approximately 250 g. per chick in four weeks) and lower dietary levels of potassium again resulted in retarded growth. These results demonstrate that with a liberal phosphorus intake, the potassium requirement for growth was lower than with a marginal or suboptimum phosphorus intake.

Efficiency of feed utilization correlated well with rate of body weight gain. Maximum efficiency was attained on the low phosphorus diets with a minimum of 0.24 per cent of potassium in the diet and on the high phosphorus diets with a minimum of 0.20 per cent of potassium in the diet.

Chicks receiving the diets containing 0.60 per cent of phosphorus required 0.16 per cent of dietary potassium for maximum bone calcification, whereas those receiving 0.35 per cent of dietary phosphorus required 0.20 per cent of dietary potassium for this function. The lower levels of dietary potassium (0.12 and 0.14 per cent) caused the amount of inorganic phosphorus in the blood plasma to be depressed as much as 30 or 35 per cent. No similar depression of plasma calcium was observed, indicating that the effect of potassium on bone calcification was mediated through its influence on phosphorus metabolism rather than on calcium metabolism. The results obtained in the two chick experiments were nearly identical. The fact that the two experiments included wide variations in dietary calcium strengthens the theory that calcium was not influencing the potassium requirement.

Data were also presented to indicate that the decreased bone calcification on low potassium intakes could not be explained on the basis of retarded growth or retarded development. Chicks making widely different rates of gain were often found to have similar bone ash values.

The effect of biotin on the development and relief of symptoms of potassium deficiency in chicks was also studied by Gillis. This investigation was prompted by the reported observation (S. G. Smith, *Science* **100,** *389 (1944)*) that paralytic symptoms in dogs ascribed to biotin deficiency responded to potassium therapy (W. R. Ruegamer, C. A. Elvehjem, and E. B. Hart, *Proc. Soc. Exp. Biol. Med.* **61,** *234 (1946)*). Symptoms of paralysis in calves fed synthetic diets could be cured with either potassium (oral) or biotin (subcutaneous) administration (R. J. Flipse, C. F. Huffman, C. W. Duncan, and F. Thorp, *J. Animal Sci.* **7,** *525 (1948)*). Gillis found no benefit in chicks suffering from potassium deficiency when supplied with as much as 1.1 micrograms of biotin per gram of diet, which is ten times the normal requirement.

In the rat experiment reported by Gillis graded amounts of potassium were added to basal diets containing 0.2, 0.3, and 0.4 per cent of phosphorus. The rat required 0.19 per cent of dietary potassium for optimum growth when the diet contained 0.2 per cent of phosphorus, whereas only 0.16 per cent of potassium was needed to produce optimum growth at the 0.3 and 0.4 per cent dietary phosphorus levels. The effect of potassium on bone calcification was also observed in the rat, although the results indicated a less striking influence. Gillis reasoned that this might be expected since a deficiency of bone-forming elements is much more serious in the very young chick than in the weanling rat.

That a potassium deficiency would occur under practical poultry feeding conditions

is unlikely. Even the increased requirement for potassium on phosphorus-low diets, as demonstrated by Gillis, would likewise be met under practical conditions. These observations are therefore of no practical significance, but they are important in that they extend the knowledge concerning the vital role of potassium in animal physiology.

Potassium was earlier demonstrated to be concerned with the function of certain phosphorylating enzymes by P. D. Boyer, H. A. Lardy, and P. H. Phillips (*J. Biol. Chem.* **149,** *529 (1943)*) and by D. Nachmansohn and H. M. John (*Proc. Soc. Exp. Biol. Med.* **57,** *361 (1944)*). The work of Gillis indicates that potassium is also concerned with phosphorus metabolism in connection with bone calcification.

PROTECTION AGAINST RADIATION DAMAGE WITH CYSTEINE

An effective method for therapy of radiation sickness assumes unusual importance and urgency in view of the turbulent world situation. The possibility exists that large numbers of our population may be subjected to radiation damage. In the present state of knowledge, little can be done for these individuals aside from general supportive and symptomatic therapy, once the radiation has been received. While such treatment may be very beneficial and may represent the difference between life and death in those individuals who have received marginal amounts of radiation, it offers little hope for those who have received larger doses. Lack of adequate specific therapy for radiation sickness is not surprising since so little is known about the mechanism by which radiation causes tissue damage and the metabolic derangements which result.

Many methods and substances have been used in attempts to influence, favorably, radiation sickness. Either folacin or pyridoxine will prolong survival in irradiated mice, although neither has an effect on the characteristic leukopenia (*Nutrition Reviews* **6,** *336 (1948)*). Some of the naturally occurring flavones have been reported to protect against hemorrhage and to reduce mortality in irradiated dogs. Ascorbic acid did not produce a similar beneficial effect, although there was some indication that it might potentiate the effect of the active flavones (*Ibid.* **7,** *317 (1949)*). Desoxycorticosterone acetate has been reported to modify, favorably, certain aspects of the pathologic picture which develops following radiation (*Ibid.* **6,** *336 (1948)*), although neither cortisone nor adrenocorticotropic hormone prolonged survival in irradiated mice (W. W. Smith, F. Smith, and E. C. Thompson, *Proc. Soc. Exp. Biol. Med.* **73,** *529 (1950)*).

Less information is available concerning the use of such substances in radiation sickness in man. Folacin has been shown to have some effectiveness in radiation leukopenia (*Nutrition Reviews* **4,** *11 (1946)*). Many radiologists have used pyridoxine in radiation sickness with apparent symptomatic benefit.

H. M. Patt and associates (*Science* **110,** *213 (1949)*) found that cysteine is highly effective in protecting rats against lethal x-irradiation. This group at the Argonne National Laboratory has extended its work by studying the effect of cysteine on the peripheral blood of irradiated rats (Patt, D. E. Smith, and E. Jackson, *Blood* **5,** *758 (1950)*).

The formed elements of the blood, particularly the leucocytes, are sensitive to radiation and should be good indicators of the biologic effectiveness of protective substances. Control and experimental rats were irradiated simultaneously, receiving 800 r total body radiation in a single exposure. This dosage produced 66 per cent mortality within seventy days in control animals as contrasted with 22 per cent mortality in

similar animals which received 950 mg. per kilogram of cysteine intravenously five minutes before irradiation. Total leucocyte, erythrocyte, reticulocyte, hemoglobin, hematocrit, and leucocyte differential determinations were done on tail blood before and at intervals following irradiation. Nonirradiated controls were also examined.

In irradiated control rats the white blood elements showed a maximal depression four days after exposure. Recovery was evident in twenty-one days and essentially complete in forty days. Similar animals treated with cysteine also showed a maximal leucocyte depression at four days but the depression was less severe and recovery began earlier (ten days). Both the granulocytes and lymphocytes were involved in the total leucocyte depression and both showed more rapid recovery in irradiated rats which were pretreated with cysteine. The granulocytes seemed to recover somewhat more quickly than the lymphocytes.

The number of circulating erythrocytes in the irradiated control rats fell sharply ten days after exposure. Maximal anemia occurred at twenty-one days; recovery began at twenty-eight days, and was essentially complete at forty days. A comparatively small decrease in erythrocytes was seen at ten days in irradiated rats pretreated with cysteine. The erythrocyte level was rapidly restored in these animals. Recovery began at fourteen days and was complete at twenty-eight days. Hematocrit and hemoglobin changes generally paralleled the erythrocyte changes. A severe depression of the reticulocytes was observed in all irradiated animals four days after exposure but recovery occurred significantly earlier in the rats receiving cysteine.

From these results and previous ones there seems to be little doubt that cysteine can confer a significant amount of protection against the biologic effects of radiation. There is, however, little to indicate how it may be functioning. Cysteine is an amino acid which can be utilized in protein metabolism, yet its mode of action against radiation is probably not nutritional since its oxidized counterpart, cystine, is not effective (Patt *et al.*, *loc. cit.*).

It should also be noted that cysteine was ineffective when given shortly after irradiation. Apparently this substance must be in the blood and tissues at the time of radiation to influence, perhaps by the action of its -SH groups, some deleterious biologic reaction activated by the radiation.

Vitamin B_{12} has a potent influence on blood formation under certain circumstances and there is apparently some relationship between it and the sulfur-containing amino acids (*Nutrition Reviews* **8,** *301 (1950)*), therefore it was logical that this vitamin might be tried against radiation damage.

R. E. Carter, E. Busch, and V. Strang (*Blood* **5,** *753 (1950)*) subjected rats to 400 r of roentgen rays and followed the peripheral blood and bone marrow changes with and without vitamin B_{12} treatment. This dose of radiation was sufficient to produce marked depression in the circulating leucocytes but only a slight depression in the erythrocytes. Only one animal died from effects ascribed to radiation and this death was in a group receiving vitamin B_{12}. The vitamin was given intramuscularly in a single dose of 4.0 micrograms thirty minutes after irradiation or as multiple doses of 0.3 microgram daily for twenty-one days beginning thirty minutes after irradiation. No significant effect of the vitamin B_{12} supplements was observed on either the leucocytes or erythrocytes when compared with irradiated control animals. Likewise there were no significant differences in the bone marrow between animals with or without the vitamin.

Vitamin B_{12} had no favorable influence on the hematologic changes of radiation sickness under the circumstances of this experiment. What effect the vitamin might have had if it had been given prior to radiation, or what effect it might have had on mortality with larger doses of radiation cannot be predicted.

The urgent need for further investigations to find substances capable of conferring protection against the effects of radiation is clearly indicated. In particular, agents are needed which would be of benefit in individuals who had already been exposed to radiation. Substances which are effective only if given prior to radiation would be of little practical help in protecting our population in case of atomic attack.

THERAPY OF POLYCYTHEMIA

Much can be done to relieve discomfort and prolong life in patients with polycythemia vera, a condition in which there is an excessive production of red blood cells. However, the etiology of this disease is completely obscure and therapy is nonspecific and symptomatic.

E. C. Bell and P. L. Davis (*Am. J. Clin. Path.* **20,** *839 (1950)*) report the apparent successful use of a folacin antagonist in a patient with this disease. The patient, a 60-year-old female, had been suffering for several years with hypertensive and arteriosclerotic heart disease. In March 1949 the diagnosis of polycythemia vera was made, as evidenced by an abnormally high red blood cell count and elevated hemoglobin. Sternal bone marrow examination showed hyperplasia of all cellular elements and apparently ruled out the possibility that the peripheral blood changes were secondary to the heart disease. "Other studies" were compatible with a diagnosis of polycythemia vera.

The patient was given three courses of 4-amino-pteroylaspartic acid by intramuscular injection. The first course consisted of six daily injections of 20 mg. each. After a rest period of one week, 40 mg. were given daily for three days, following which 20 mg. were given daily for four days. At this time the patient was discharged from the hospital and was given six injections of 20 mg. each per week until 240 mg. had been received. The total dose of the folacin antagonist was 560 mg.

The red blood cell count and hemoglobin began to fall after seven days of treatment and continued to fall after treatment was stopped. The erythroblastic series in the bone marrow also decreased in number. Three months after therapy was started the erythrocyte count and hemoglobin concentration were entirely normal. Four months later, when the patient was again seen, the blood picture was about the same as before treatment. In the article no mention is made as to whether antifolacin therapy was tried again. The patient had no discomfort or ill effects from the antagonist. No mention was made of any effect the antagonist may have had on the leucocytes.

In an addendum to the article the authors state that they have since had 2 additional patients with polycythemia vera in whom they have been able to maintain a state of remission for six months with 4-amino-pteroylaspartic acid.

It is not surprising that a folacin antagonist might have some effect on red cells. In many of the early experiments on rats and other animals which were suffering from leukopenia due to folacin deficiency, anemia occurred with a variable frequency (S. S. Spicer, F. S. Daft, W. H. Sebrell, and L. L. Ashburn, *Pub. Health Reports* **57,** *1559 (1942)*). The effect of folacin in pernicious anemia and other macrocytic anemias is well known. It will be interesting to note whether patients with polycythemia vera eventually develop a resistance to the folacin antagonist as do patients with leukemia.

The present study does not of course establish that 4-amino-pteroylaspartic acid is an effective agent in polycythemia vera. Spontaneous remissions are known. However, the data are sufficiently convincing to warrant further trial.

W. Dameshek (*J. Am. Med. Assn.* **142,** *790 (1950)*) has published an interesting survey of what is known about polycythemia vera. He mentions various methods which have been used in the treatment of this disease such as arsenic, benzol, nitrogen mustard, phenylhydrazine, spray radiation, and radioactive phosphorus. The method which he prefers is periodic venesections combined with an iron-deficient diet.

The patients are treated by removing 500 ml. of blood twice weekly until the blood picture is within or slightly below the normal range. This procedure usually takes two to four weeks and it not only reduces the red blood cell mass but usually induces a state of iron deficiency in the bone marrow so that the erythrocytes produced are small and hypochromic. The low iron diet is begun at the end of the course of venesections.

The patient is advised to curtail sharply the intake of foods rich in iron such as red meats, liver, and eggs; certain green vegetables, notably spinach, string beans, and Swiss chard; brown cereals (hominy, oatmeal, whole wheat, and bran); and shell fish, including oysters, clams, and lobsters. To maintain the protein requirement the patient is asked to drink two to four glasses of milk daily, eat fish two or three times weekly, chicken or other fowl twice weekly, lamb or veal once or twice weekly, and red meat not more than once each week.

This reduction in iron intake leads to an insufficient amount of iron for hemoglobin synthesis with the result that the red cells produced continue to be smaller than normal and so occupy less space in the circulation than do normal cells.

The observations indicate that the remission induced by venesections persists for a longer period when the dietary iron intake is restricted than when the patient is on a normal diet. A clinically satisfactory remission may be maintained for three to eighteen months or even longer. When the symptoms recur a few venesections are usually sufficient to control the disease. In some cases patients on this regimen develop a brilliant red glossitis. There is also the disadvantage that periodic venesections are required. In elderly patients the venesections may precipitate circulatory collapse due to the rapid change in circulatory dynamics. The method does not correct the high platelet count so that there is a tendency toward thromboses.

Despite the side effects mentioned above, Dameshek has found the method satisfactory for at least 9 of 10 patients with polycythemia vera and it is sufficient to control the disease for a few to many years. Some patients have been under observation for ten to fifteen years and are in as good health as comparable persons of the same age group.

This method, like the folacin antagonist, is not specific therapy but until the etiology of the disease is discovered and specific treatment can be devised, it offers a way to relieve discomfort and prolong life.

UTILIZATION OF NONPROTEIN NITROGEN

One of the definitions frequently given to beginning students in physiological chemistry is that proteins are organic compounds containing nitrogen in physiologically available form. This nutritional aphorism is misleading for it is known that there are nonprotein compounds whose nitrogen appears to serve certain needs of the mammalian organism.

Many years ago W. Völtz (*Biochem. Z.* **102,** *151 (1920)*) demonstrated that adult sheep restricted to a nitrogen-poor ration could maintain nitrogen balance in those periods in which 30 g. of urea were added to 500 g. of chopped oat straw, 400 g. of molasses, and 150 g. of cooked potato starch. Upon this dietary regimen, body weight was maintained. In similar experiments with

lambs there was a substantial gain in body weight when urea was added to the basal ration and it appeared that under these conditions urea assumed the role of dietary protein.

In a later investigation with calves, E. B. Hart, G. Bohstedt, H. J. Deobald, and M. I. Wegner (*J. Dairy Sci.* **22,** *785 (1939)*) demonstrated that the nitrogen from urea and from ammonium bicarbonate could be used during growth for at least a partial supply of protein, the most probable explanation being that growth of the bacteria in the rumen was greatly stimulated by these simpler forms of nitrogen and that the bacterial protein in turn was digested in the fourth stomach and intestine thus providing the calf with the amino acids required for growth.

That the above explanation is probably valid was shown by E. Studt (*Z. f. Tierzüch. u. Züchtungsbiol.* **44,** *253 (1939)*) in long-continued studies with sheep which were fed a nitrogen-poor basal ration supplemented with various ammonium salts. Equating the food nitrogen with the nitrogen in the increments in body weight and wool, it was apparent that protein has been synthesized from sources of nitrogen other than that in the food. Ninety per cent of the nitrogen in the contents of the paunch was protein which was more digestible than the food protein and far more abundant; the microorganisms in the paunch, growing on mediums with comparatively simple compounds of nitrogen, apparently contribute their cellular protein in turn to the nutrition of the host.

Studies on adult sheep (L. E. Harris and H. H. Mitchell, *J. Nutrition* **22,** *167 (1941)*) maintained on a low-nitrogen basal ration indicated that addition of urea to the diet resulted in improvement of appetite just as when casein was added, and that a distinct improvement in the digestibility of the dry matter of the basal ration followed supplementation with urea. Nitrogen balance always improved with urea and even storage could be induced when similar dietary adjustments were made with lambs (Harris and Mitchell, *Ibid.* **22,** *183 (1941)*); nearly normal growth was secured when urea was fed, and no evidence of toxicity was observed over a period of a hundred and ten days. The carcasses of the lambs fed urea were examined for dry matter, nitrogen, and gross energy and it was concluded that no marked differences in composition were produced by the different levels of urea fed.

That mammals with simpler gastrointestinal tracts than possessed by the ruminant do not utilize nonprotein nitrogen is indicated (M. Kriss and L. F. Marcy, *J. Nutrition* **19,** *151 (1940)*) by the observation that urea fed with a basal ration to adult rats is nearly quantitatively recovered from the urine and feces. The rats were unable to retain appreciable amounts of nitrogen from the urea and it appears that rats cannot utilize this nitrogen for the synthesis of protein.

A different approach has been made by feeding rats on a purified diet containing 15 per cent casein and ammonium citrate containing N^{15} (D. Rittenberg, R. Schoenheimer, and A. S. Keston, *J. Biol. Chem.* **128,** *603 (1939)*). The proteins prepared from the liver, intestine, kidney, and muscle of these rats contained N^{15} which also appeared in the glutamic acid and glycine prepared from liver and intestine protein, in the arginine from all the proteins, and in the aspartic acid from liver protein. It was claimed that the foregoing results demonstrate the ability of the rat to utilize ammonium nitrogen for amino acid formation.

A study of the utilization of nonprotein dietary nitrogen for the synthesis of nonessential amino acids in the growing rat (W. C. Rose, L. C. Smith, M. Womack, and M. Shane, *J. Biol. Chem.* **181,** *307 (1949)*) bears on the present discussion. Young rats grew slowly when restricted to a ration in which the nitrogen was provided by the essential amino acids alone. When the nonessential amino acids were added, good growth occurred. When diammonium citrate or L-glu-

tamic acid containing nitrogen equivalent to that provided by the nonessential amino acids replaced them, excellent growth was observed. Neither glycine nor urea was as effective as diammonium citrate or L-glutamic acid in providing nonprotein nitrogen for growth.

In another experiment, rats were given a ration producing a minimal positive nitrogen balance; when diammonium citrate was added, a marked retention of nitrogen was observed. Again, 21-day-old rats were given a diet containing the essential amino acids at a 4, 8, and 12 per cent level (H. A. Lardy and G. Feldott, *J. Biol. Chem.* **186,** *85 (1950)*); at each protein level, supplementation of the ration with diammonium citrate stimulated better growth than with protein alone. No added growth was produced by adding sodium citrate, showing that it is the ammonium nitrogen which is active. The D-amino acid oxidase content of the liver of these rats was proportional to the level of protein in the diet; however, when supplemental nitrogen in the form of diammonium citrate was added, the titer for D-amino acid oxidase remained unchanged.

The current evidence supports the view that not only can nonprotein nitrogen indirectly support nitrogen metabolism in ruminants, but also that in the laboratory rat, the nitrogen of ammonium salts and certain other compounds can replace amino acid nitrogen in metabolism and growth.

METABOLISM OF PHYTIN LABELED WITH P^{32}

Generally it has been assumed that the organically bound phosphorus in phytin (phytic acid or its salts) is not readily available as a source of phosphorus for chicks and turkey poults, and that the intact phytin molecule is eliminated largely unchanged in the feces. Vitamin D is known to be effective in improving the availability of phytin phosphorus. The mode of this action is not clearly understood, and it has been speculated that the vitamin may act through enhancing the activity of the enzyme phytase. This enzyme is present in cereal grains and animal tissues and it is believed that it is capable of hydrolyzing phytin to inositol and phosphoric acid.

Whether phytin phosphorus actually participates in bone formation has been clarified by tracer studies at the Connecticut Agricultural Experiment Station, Storrs, Connecticut (E. P. Singsen, L. D. Matterson, and A. Kozeff, *Poultry Sci.* **29,** *635 (1950)*). These workers fed phytin containing the phosphorus isotope P^{32} to turkey poults and demonstrated that significant amounts of radioactivity accumulated in the bone ash.

The radioactive phytin was synthesized biologically by growing corn in gravel culture that was fertilized with a nutrient solution containing P^{32}-labeled monobasic potassium phosphate. The phytin was isolated from the corn and fed as calcium phytate at a 1 per cent level in a phosphorus-low basal diet of the following composition: special white corn meal 69; casein 12; gelatin 3; primary grown yeast 4; bagasse 4; liver meal 3; wheat germ oil 1; calcium carbonate 2; potassium chloride 0.50; sodium chloride 0.50; and manganese sulfate 0.0025 per cent. The diet was fortified with vitamins and the vitamin D content was varied to determine its influence on the utilization of phytin phosphorus.

One group of 6 poults received the diet with no supplemental vitamin D; a second group of 6 poults received the same diet supplemented with U.S.P. cod liver oil; and a third group of 6 poults received irradiated 7-dehydrocholesterol as a source of vitamin D. The amount of vitamin D supplement for groups 2 and 3 was the same, i.e., 80 A.O.A.C. (American Organization of Agricul-

tural Chemists) chick units per 100 g. of feed.

The basal diet was fed to the day-old poults for one week prior to addition of the radioactive calcium phytate; the diet containing the latter was then fed for a two-week experimental period. To determine the rapidity with which the isotope might enter the tissues, one poult from each group was killed one hour after the radioactive phytin was fed. Significant amounts of radioactivity were present in blood, down, and muscle tissue within one hour after the radioactive material was fed.

Subnormal bone (tibia) ash values were found in all of the poults that were on the experimental diet for two weeks. The poults that received supplemental vitamin D had significantly higher bone ash values than those receiving no supplement. The bone ash values indicated that vitamin D from 7-dehydrocholesterol promoted better utilization of phytin phosphorus than did the vitamin D from cod liver oil.

Radioactivity measurements on wet-ashed bone indicated that the phytin phosphorus was being deposited in the bony structure and was not merely located in the organic matrix. The data supported the conclusion that the action of vitamin D in promoting better phosphorus utilization represented an influence of the vitamin on the phytin phosphorus rather than simply in enhancing the utilization of the nonphytin phosphorus of the ration. For example, radioactivity counts per minute on tibia ash for the group not receiving vitamin D supplement were 524, contrasted with counts of 936 and 1155 for the groups supplemented with cod liver oil and irradiated 7-dehydrocholesterol, respectively.

On the basis of their results, the authors favored the view that the phosphorus in phytin takes part in exchange reactions and that these reactions are partially under the influence of vitamin D. Thus, in the absence of vitamin D, they believe phytin removes as much phosphorus from the body as it brings in, whereas under the influence of vitamin D the body is able to replace part of the phosphorus of phytin with some other radical and use the phosphorus so released. Bone calcification studies with rats have indicated that such an exchange reaction explains the rate of turnover of radioactive phosphorus and the dynamic behavior of calcified tissues (W. F. Neuman and R. F. Riley, *J. Biol. Chem.* **168,** *545 (1947)*; M. Falkenheim, Neuman, and H. C. Hodge, *Ibid.* **169,** *713 (1947)*).

Unfortunately the Connecticut workers did not obtain balance data in their studies with turkey poults. It would be important to know to what extent the phosphorus balance was being influenced and whether there was improved retention of phosphorus as a result of vitamin D action.

In some earlier studies H. Schneider and H. Steenbock (*J. Biol. Chem.* **128,** *159 (1939)*) concluded that on phosphorus-low diets, vitamin D induced utilization of phosphorus by bone at the expense of the phosphorus in the soft tissues. In this connection it should be pointed out that calcium phytate was reported to be almost completely unavailable for chicks even in the presence of vitamin D (*Nutrition Reviews* **8,** *317 (1950)*).

The results obtained by the Connecticut workers do, however, emphasize that the phosphorus in phytin is labile and that it is available to the animal for bone formation. Whether, under such conditions, there is actually a net gain of phosphorus to the animal remains to be established.

GLUTAMIC ACID AND MENTAL FUNCTIONS

Recent articles in popular magazines have inferred that the amino acid known as glutamic acid has special therapeutic properties for the treatment of various mental disorders. Glutamic acid has been referred to as "brain food." It has been claimed to result in an increase in the I.Q. of mentally retarded children and adults. These claims do not stand up under careful examination and are a good example of premature, sensational publicity based on inadequately controlled studies. Such claims usually result in more harm than good because of the false hopes they create.

Biochemically, there is at present no positive evidence to indicate a direct effect of glutamic acid administration on the brain in the intact organism. A direct influence would necessitate entry of this compound into the brain tissues. In experimental animals this does not take place. The uptake of glutamic acid by the tissues of several organs (brain, muscle, liver, kidney) was studied by H. Waelsch (*Lancet* **II,** *1 (1949)*) after intravenous administration of large amounts of the acid. No significant increase was found in the concentration of glutamic acid in the brain of mice and rats thirty minutes after the injection. In the liver of the rat the glutamic acid content was about doubled, in the kidney quadrupled within twenty minutes.

Glutamic acid is one of the amino acids which occurs in fairly large amounts in many animal and vegetable proteins. In the majority of clinical studies little or no attention has been paid to the amount of glutamic acid provided by the dietary proteins. In addition, glutamic acid is readily synthesized in the animal and human body.

Patients receiving supplements of glutamic acid are, in fact, expected to be benefited by addition to the diet of relatively small amounts of a substance which is provided, in relatively large quantities, in the dietary protein and which is synthesized in the body. It would appear more logical to consider the level of glutamic acid in the blood than to rely on the size of the supplement taken by mouth. There is no evidence that mentally deficient children are able to utilize or to synthesize glutamic acid less efficiently than children at the higher levels of intelligence. Waelsch (*loc. cit.*) observed that concentrations of glutamic acid in the blood plasma of 19 epileptic and 13 nonepileptic persons were identical.

As elaborated by Waelsch (*loc. cit.*), the biochemist's work on the metabolism of glutamic acid and its relation to the nervous system suffers from severe limitations. For one, most of the diseases of the nervous system have no known counterpart in the lower animals, the biochemist's preferred subjects. The biochemist concerned with the fundamental mechanisms responsible for the abnormal function of the nervous system has to rely largely on clinical material and is handicapped by the impossibility of adequate experimentation on animals.

Studies on the effects of glutamic acid on animal intelligence have not been lacking but were concerned with the behavior of normal animals maintained on different levels of glutamic acid. F. T. Zimmerman and S. Ross (*Arch. Neurol. Psychiat.* **51,** *446 (1944)*) reported that glutamic acid enhanced the ability of white rats to learn a simple maze. E. Stellar and W. D. McElroy (*Science* **108,** *281 (1948)*) commented that the difference seems to have been due to the unusually poor performance of the control animals rather than an exceptional performance of the supplemented group. K. Albert and C. J. Warden (*Ibid.* **100,** *476 (1944)*) observed that rats supplemented with glutamic acid learned to master more difficult tasks than did the control animals.

Except for these first two animal investigations, all subsequent research yielded essentially negative results. M. H. Marx

found no effect on learning or re-learning a modified Stone multiple-T water maze (*Am. Psychologist* **1,** *273 (1946)*); *J. Comp. Physiol. Psychol.* **41,** *82 (1948)*). H. C. Hamilton and E. B. Maher (*Ibid.* **40,** *463 (1947)*) reported that supplementary feeding of glutamic acid to young adult white rats did not result in a higher level of performance.

The conflicting findings led Stellar and McElroy (*loc. cit.*) to duplicate the procedures of Zimmerman and Ross whose work of 1944 initiated the controversy. The principal remaining difference was in the strain of animals. The results indicated no statistically significant difference in the performance of the experimental animals fed glutamic acid and the controls. It took both groups 5.1 trials to meet the accepted criterion of satisfactory performance. Since both groups learned the maze about as fast as Zimmerman and Ross' supplemented animals and since the task may have been too easy to differentiate between the control and supplemented animals of the strain used by the Johns Hopkins investigators, the animals were continued on the dietary regimen and were tested on two more difficult mazes. Even under these conditions there was no significant difference in performance.

M. H. Marx (*J. Comp. Physiol. Psychol.* **42,** *313 (1949)*) tested the possibility that addition of large amounts of glutamic acid might result in a greater differentiation between the performance of the experimental and the control animals than he was able to establish previously. The diet of the experimental animals contained 10 per cent of glutamic acid. The differences between the experimental and control animals were slight, statistically nonsignificant, and their direction was in favor of the control group. In a subsequent experiment, reported in the same paper, Marx attempted to increase the control of the diet composition and to provide a still greater glutamic acid differential, using a 20 per cent glutamic acid supplement. Even then there were no statistically significant differences in the mean number of trials necessary to run the maze perfectly, in the total time, or in the total number of errors.

Another study on the effects of glutamic acid on maze learning was made by P. B. Porter and A. C. Griffin (*J. Comp. Physiol. Psychol.* **43,** *1 (1950)*). Whereas previous investigators were concerned with the effect of high intakes of glutamic acid, and improved learning, Porter and Griffin examined the hypothesis that low intakes (50 versus 560 mg. of glutamic acid per day) would affect adversely the maze learning. Their second concern was to investigate whether differences in the glutamic acid content of the diet would modify the effects of electroconvulsive shocks. There was no reliable difference either in the mean swimming time on the 7-unit water maze after ten trials or in the number of errors. Also, the added glutamic acid did not serve as an effective "shield" against the effects of a series of ten electroconvulsive shocks on maze learning and did not promote a more rapid recovery during the relearning of the maze.

Except for the initial two studies, both carried out at Columbia University and using the same basic diet, not simultaneously analyzed for the glutamic acid content, and the same strain of animals, all the subsequent studies on the level of glutamic acid feeding and animal intelligence were essentially negative. Some of the experiments followed closely the original work. Other studies explored supplementation levels and types of performance different from those used in the initial investigations. It is well established by now that under a variety of experimental conditions the level of glutamic acid in the diet does not affect significantly the rats' ability for maze learning and problem solving.

Clinical research on the potential effect of glutamic acid on intelligence was stimulated by the incidental observations of increased

alertness made in a study on the treatment of epilepsy (J. C. Price, H. Waelsch, and T. J. Putnam, *J. Am. Med. Assn.* **122,** *1153 (1943)*; *Nutrition Reviews* **5,** *174 (1947)*; **6,** *342 (1948)*). Encouraging results were obtained (Waelsch, *loc. cit.*) in a study on mental defectives made available by the New York City's Bureau for Retarded Children. No selection of subjects was made, except that children with a history of a convulsive disorder were excluded. The study has not yet been reported in full. Although the clinical investigations were devoted principally to the problem of mental deficiency, more recently the use of glutamic acid was considered, without a good rationale and adequate experimental design, in the treatment of schizophrenia (J. R. Ewalt and E. I. Bruce, *Texas Rep. Biol. Med.* **6,** *97 (1948)*; H. Kitzinger, D. G. Arnold, R. W. Cartwright, and D. Shapiro (reprinted from *Rorschach Res. Exch. and J. Projective Techniques* **13** (*No. 2*) (*1949*)).

Two studies (F. T. Zimmerman, B. B. Burgemeister, and T. J. Putnam, *Arch. Neurol. Psychiat.* **61,** *275 (1949)*; *Am. J. Psychiat.* **105,** *661 (1949)*) dealt with the effect of glutamic acid on the intelligence of patients with mongolism, a serious and not well understood form of mental deficiency. Both studies reported positive results, with average gains of 8 months of mental age, determined by the Stanford-Binet scale of intelligence, in six months of glutamic acid treatment. These studies, like the earlier work of the Columbia group, suffer from lack of rigorous control observations. This is unfortunate, because the clinical aspects of the studies on glutamic acid treatment of mental retardation appear to be carried out carefully (Zimmerman, *Quart. Rev. Psychiat. Neurol.* **4,** *263 (1949)*).

Perhaps the incidental observations on the effect of glutamic acid on physical growth provide the best argument for inclusion of a control group. The authors noted the irregularity of growth of children with mongolism but did not draw the logical conclusion that this irregularity alone, plus factors in the environment and other influences, such as seasonal variations in growth, could account for all or a large part of the effect ascribed to glutamic acid. Retardation of growth is most conspicuous during the early years of life, when complete arrest of growth for many months may be observed. Between 3 and 13 years of age the rate of growth may be normal. Only inclusion of a control group, matched carefully with the experimental group and differentiated only by the glutamic acid intake, can extricate the influence of the variable under study from the effect of other factors.

The data accumulated during five years of study on 252 nonmongoloid mentally deficient individuals were summarized by F. T. Zimmerman and B. B. Burgemeister (*Education* **70,** *410 (1950)*). Following six months of glutamic acid therapy the group showed average gains of ten months of mental age and 5.1 points in the intelligence quotient according to the Stanford-Binet test. The mean changes are statistically highly significant. Biologically, the average gains appear impressive. It should be noted that a normal child's average gain in six months of life is six months of mental age. Twenty-three individuals with initial intelligence quotients at the upper end of the defective range and 12 with an I.Q. at the borderline level of intelligence were selected for special statistical study (*New York State J. Med.* **50,** *693 (1950)*). Twelve patients were considered to have improved sufficiently to become—from the standpoint of conventional prerequisites of general intelligence—capable of economic self-sufficiency. These cases certainly would deserve further careful study to determine the permanency of the change in intellectual level and the development of personality characteristics involved in holding a job.

Whereas Zimmerman reported continuing improvement after the glutamic acid therapy was discontinued, K. Albert, P. Hoch, and

H. Waelsch (*J. Nerv. Ment. Dis.* **104,** *263 (1946)*) noted that when supplementation was discontinued and/or glutamic acid was replaced by a lactose placebo, the I.Q. dropped back almost to the original level.

The greatest need at present is for carefully controlled investigations. A pilot study of this kind was carried out by W. J. Kerr and S. A. Szurek (*Pediatrics* **5,** *645 (1950)*), indicating no effect of glutamic acid supplementation on measured intelligence. H. G. Loeb and R. D. Tuddenham (*Ibid.* **6,** *72 (1950)*) studied institutionalized feebleminded individuals, ranging in age from 6 to 29 years, free of epilepsy or other obvious neurologic involvements. The subjects were divided into the control group (N = 15) and the experimental group (N = 18), approximately equated on the basis of sex, age, and intelligence as measured by the Stanford-Binet test. For sixteen weeks the experimental subjects were getting 24 g. of monosodium glutamate daily. A battery of psychologic tests was administered before the treatment was instituted and again at the end of the experimental period. The findings indicate a lack of beneficial effect of glutamic acid on intelligence or performance capacity.

A well-controlled study on the influence of glutamic acid on performance was carried out by D. G. Ellson, P. R. Fuller, and R. Urmston (*Science* **112,** *248 (1950)*). The subjects were two groups of 30 children, resident in a state school. The experimental and the control groups were closely matched by pairs for age and intelligence. The experimental period extended from September 1947 to June 1948. Five psychologic tests were administered at the start, middle, and end of the experimental period. The only statistically significant changes occurred in the test of coordination, both in the time and the error score but in the opposite directions. The experimental (supplemented) group was faster but less accurate than the control subjects.

The mean gain in I. Q. of 5.2 points in the experimental group was identical with the value reported by Zimmerman and Burgemeister (*Education, loc. cit.*) for their group of 252 mentally retarded individuals. Though in the study by Ellson *et al.* there was a lapse of ten months between the two administrations of the same form of the Stanford-Binet test, there was an improvement of 3.3 points in the mean I.Q. value in the control group. The maximal gains of the experimental subjects was 21 I.Q. points, in the control group 19 points.

Negative results were obtained also in the study of T. L. McCulloch (*Am. J. Ment. Deficiency* **55,** *117 (1950)*) on the effects of glutamic acid feeding on cognitive abilities of institutionalized mental defectives. The study involved concurrent use of an experimental (N = 22) and a control group (N = 21), equated in respect to chronologic age, mental level, and diagnosis, living under much the same environmental conditions and receiving a comparable diet. The experimental feeding extended over six months. There was no evidence of a beneficial effect of supplementary feeding of glutamic acid on measured intelligence (Stanford-Binet test) of institutionalized mentally defective children. The children with higher levels of intelligence were tested on several other mental tests but no significant difference was established between the glutamic acid and the control group.

Attention was directed to cases which deviated most in their retest scores on the Stanford-Binet test. Of special interest are two sets of male identical twins. Before the start of the experimental regimen the 6-year-old twins had an I.Q. of 49 and 57, respectively; after six months the twin receiving glutamic acid supplement gained eight months in mental age while his twin control gained eleven months. In the 8-year-old twins, with I.Q.'s of 63 and 62, the gains were eight and seven months. Thus for both sets of twins relatively large increments in test scores were observed, unrelated to the dietary status of the subjects during the

control period. A certain amount of intra-individual fluctuation in test scores obtained for a group of subjects is always present. If the effect of random fluctuations and nondietary factors would not be taken into account, the gains would be attributed, incorrectly, to glutamic acid feeding.

A thorough review of the literature brings one to the conclusion that glutamic acid is likely to follow the same dreary career experienced by numerous other "brain foods" recommended in the past, such as the phosphates of the 'twenties. The underlying reasoning, not found in the actual papers, is as tempting as it is logically unconvincing: there is lots of the substance in the brain, it *must* have something to do with the brain function; the more, the better; let's feed it. The fact that glutamic acid is already contained in the food and is synthesized by the organism has been largely not considered by the clinicians.

EFFECT OF PANCREATECTOMY ON SYNTHESIS OF PLASMA PHOSPHOLIPID

The liver is importantly concerned in the metabolism of fat within the animal body and has a significant role in the regulation of phospholipid circulating in the blood plasma. Evidence has accumulated indicating that plasma phospholipid is chiefly derived from sources of synthesis within the liver and is, in addition, degraded and removed by this organ (*Nutrition Reviews* **5,** *135* (*1947*); **8,** *147* (*1950*)).

That conditions or agents which influence the metabolism of fat in the liver would also indirectly produce changes in plasma phospholipid, might be anticipated. Such a condition occurs when the pancreas is removed and animals are then maintained on insulin. In this instance fatty livers develop with accumulation of large quantities of neutral fat and relative or absolute decrease in the amount of hepatic phospholipid.

W. F. Barker, K. E. Rogers, and F. D. Moore (*Arch. Surg.* **61,** *1151* (*1950*)) have investigated the effect of pancreatectomy on plasma phospholipid in dogs by determination of the specific activity of phospholipid phosphorus of plasma after administration of radioactive phosphorus.

The animals used in these experiments were mature, female mongrel dogs. They were fed diets composed of lean horse meat, sucrose, minerals, and vitamins without addition of lipotropic substances. After pancreatectomy the animals were given daily injections of protamine zinc insulin in quantities sufficient to control the diabetes resulting from removal of the gland. Liver biopsies were taken for lipid analysis at the time of initial operation, at intervals during the course of the experiment, and finally when the animals were killed.

Radioactive phosphorus was administered as isotonic disodium phosphate through a stomach tube. The test dose was approximately 0.1 millicurie per kilogram of body weight. Animals were fasted for six hours prior to the time of administration of the radioactive phosphorus. Bloods were withdrawn at intervals and the radioactivity of the plasma inorganic and phospholipid phosphorus determined.

In this study specific activities of phospholipid phosphorus are expressed as relative radioactivity of the phosphorus of phospholipid as compared with that of inorganic phosphorus at the time of complete mixing. This is referred to as the specific activity ratio.

In 11 normal dogs the maximum specific activity of phospholipid was reached in about forty-eight hours and the specific activity ratio averaged 87. Similar determinations done one and a half to five months after pancreatectomy in 13 dogs showed lower maximum specific activity ratios with a mean of 43. The difference in means is statistically significant. When phospholipid

turnover was studied in the same animals both before and after pancreatectomy the peak specific activity ratios were reduced 50 to 65 per cent by removal of the gland.

Lipid analysis of specimens of liver demonstrated that not all animals had developed fatty livers within one to five months after pancreatectomy or at the time of study of phospholipid turnover. However, specific activity ratios were similar in depancreatized dogs with fatty livers and in those without fatty livers.

The authors conclude that their data demonstrate that there is a depression of synthesis of plasma phospholipid in depancreatized dogs which presumably results from deprivation of a factor derived from the pancreas and which is effective in systems of phosphorylation in the liver. However, the published report does not take into consideration other mechanisms which may produce similar depression of specific activity of phospholipid phosphorus under the conditions of these experiments.

Of equal importance is the rate of removal of phospholipid from plasma. It would have been possible to have determined rates of phospholipid removal or breakdown by measurement of specific activities at intervals after injection of preformed radioactive phospholipid obtained from donor animals. The conclusion drawn by the authors cannot, therefore, be accepted without qualification. However, an important difference in turnover of plasma phospholipid has been demonstrated in these depancreatized animals.

Earlier observations of I. L. Chaikoff, D. B. Zilversmit, and C. Entenman (*Proc. Soc. Exp. Biol. Med.* **68,** *6 (1948)*) showed that plasma phospholipid turnover rates were normal in depancreatized dogs maintained on insulin and fed fresh pancreas. These observations combined with those of Barker and associates (*loc. cit.*) provide evidence of a factor in pancreas important in controlling phospholipid metabolism in the liver and in regulating the phospholipids of plasma. Another facet is therefore added to the ever broadening evidence that the pancreas contains or elaborates a substance essential to the utilization of lipid in the body.

RELATION BETWEEN TOCOPHEROL AND CAROTENE CONTENT OF MILK AND ITS RESISTANCE TO OXIDIZED FLAVORS

A relationship between the tocopherol content of milk fat and the ability of the milk to resist reactions that produce oxidized flavors has been shown by workers at Cornell University (V. N. Krukovsky, J. K. Loosli, and F. Whiting, *J. Dairy Sci.* **32,** *196 (1949)*). Krukovksy, Loosli, and D. A. Theokas (*Ibid.* **32,** *700 (1949)*) also observed that the type of hay and pasture fed to the cows likewise influenced the stability of the milk to oxidation.

These investigators have reported that there is a significant correlation between the tocopherol and the carotenoid content of the milk fat from four breeds of cows (Krukovsky, Whiting, and Loosli, *J. Dairy Sci.* **33,** *791 (1950)*). No significant correlation was found between the tocopherol and vitamin A content of the milk from the same cows.

Milk samples were analyzed toward the end of the pasture season, in October 1947; after five months of barn feeding, in March 1948; and again after three months of pasture feeding, in July 1948.

One hundred and twenty-eight cows were represented in the data, hence the values reported for these nutritionally important milk constituents deserve some comment. The samples of milk fat from Guernsey cows were higher in tocopherols, carotenoids, and total vitamin A content than the milk

fat of any of the other breeds. Samples from Holstein-Fresian cows were uniformly lowest in tocopherol and carotenoid contents, and Brown Swiss and Jersey samples were intermediate.

The over-all average tocopherol and total vitamin A contents, respectively, per 100 g. of milk fat, were as follows: Guernsey, 3033 micrograms and 4883 I.U.; Jersey, 2623 micrograms and 3910 I.U.; Brown Swiss, 2550 micrograms and 3968 I.U.; and Holstein-Fresian, 2220 micrograms and 3135 I.U. These results are of particular interest since it is generally believed that the total vitamin A potency of butterfat from the milk of different breeds of cows on the same ration is not decidedly different.

During the pasture seasons the samples of milk fat were 24 per cent higher in tocopherols, 55 to 71 per cent higher in carotenoids, and 42 to 60 per cent higher in total vitamin A activity than during the barn-feeding season. As already mentioned, a highly significant correlation was found between the tocopherol and the carotenoid contents of the milk fat.

Stability of the milk to oxidized flavors was tested by storing pasteurized samples for seven days at 0°C. There was a relationship between the tocopherol content of the milk fat and the ability of the milk to resist oxidized flavors. A high proportion of the samples of milk that contained less than 2500 micrograms of tocopherols per 100 g. of milk fat were unstable and developed oxidized flavors in three days. On the other hand, only a very small percentage of the samples that contained 2500 micrograms or more of tocopherol per 100 g. of milk fat developed oxidized flavors in the seven-day storage period.

V. N. Krukovsky and co-workers (*J. Dairy Sci.* **32,** *679 (1949)*) had shown earlier that promotion of oxidized flavors during storage of milk products was apparently dependent upon the stability of the tocopherol present and that the destruction of carotene and vitamin A followed that of tocopherols.

Whether the increased tocopherol content of milk high in carotenoids could be traced to increased dietary intakes of tocopherol was not determined. Presumably, pasture feeding would supply more liberal intakes of tocopherols than barn feeding, since pasture grasses are excellent sources of tocopherols as well as carotenoids. It would be of real interest to establish whether the tocopherol content of cow's milk could be increased by supplementing the ration with carotene alone.

G. H. Beck, C. H. Whitnah, and W. H. Martin (*J. Dairy Sci.* **22,** *17 (1939)*) reported that the stability of milk fat to oxidized flavors was increased by supplementing the ration with carotene concentrates during barn feeding. The Cornell investigators noted, however, that carotene concentrates may also be unusually rich in tocopherols. Some samples they analyzed contained as much as 20,000 micrograms per gram.

Milk fat is not considered a good source of tocopherol (vitamin E); vegetable oils are known to be a much richer source. The small quantity present in milk, however, apparently serves an important function as an antioxidant in the preservation of carotene and vitamin A, as well as in the prevention of the development of oxidized flavors.

INFLUENCE OF FOLACIN AND VITAMIN B_{12} ON PYRIDINE TOXICITY IN RATS

The toxic effects of pyridine in rats have been studied by several investigators. R. A. Coulson and F. G. Brazda (*Proc. Soc. Exp. Biol. Med.* **69,** *480 (1948)*) noted that pyridine feeding produced marked changes in the composition and size of the liver. J.

H. Baxter (*Am. J. Path.* **24,** *503 (1948)*) reported liver and kidney injury with calcium deposits and cirrhosis in rats fed pyridine. The former workers also observed that methionine and cystine afforded protection against pyridine toxicity, but that choline was ineffective. On the other hand, Baxter (*Bull. Johns Hopkins Hosp.* **85,** *138 (1949)*) observed that either choline or cystine alone was only slightly protective but that a combination of them afforded as much protection as methionine.

The information available to date thus suggests that methylated compounds are concerned with the detoxification of pyridine. Vitamin B_{12} and folacin are concerned with the metabolism of such methylated compounds as methionine and choline (*Nutrition Reviews* **8,** *301 (1950)*). A report by J. S. Dinning, C. K. Keith, J. T. Parsons, and P. L. Day (*J. Nutrition* **42,** *81 (1950)*), dealing with the influence of vitamin B_{12} and folacin on the metabolism of rats fed pyridine, is of considerable interest.

Weanling rats were used in the study. They were fed a diet of the following percentage composition: casein 15, sucrose 70, Crisco 10, salt mixture 2, and cod liver oil 3. The diet was supplemented with the following components (in milligrams per 100 g. of diet): thiamine chloride 0.5, riboflavin 0.5, pyridoxine hydrochloride 0.5, niacin 2.0, i-inositol 10.0, choline chloride 10.0, biotin 0.005, and vitamin K 0.025.

When pyridine was added to the diet at a level of 1 per cent, the average daily body weight gain over the thirty-day experimental period was reduced from the control level of approximately 3.7 g. to approximately 2.5 g. Vitamin B_{12} (5 micrograms per 100 g. of diet) or an antipernicious anemia concentrate from liver (5 units per 100 g. of diet) counteracted the growth-depressing action of pyridine. Folacin supplement (500 mg. per 100 g. of diet) was only partially effective in counteracting the growth depression induced by feeding pyridine, and a combination of vitamin B_{12} and folacin was less effective than vitamin B_{12} alone.

Folacin supplements under these experimental conditions appeared to induce a choline deficiency that was evidenced by reduced growth, and a yellow, fat-infiltrated liver noted at autopsy. No data, however, were given on the composition of the livers other than the liver-body weight ratios. Pyridine feeding induced enlarged livers and this liver enlargement appeared to be enhanced by folacin in the presence of vitamin B_{12}. Liver concentrate or vitamin B_{12} alone did not influence liver weight.

The authors suggest that the indicated choline deficiency was a result of accelerated methylation of the pyridine in the presence of folacin and vitamin B_{12} with a consequent loss of methyl groups from the body. The basal diet contained a limited amount of supplemental choline as previously indicated. Whether additional choline would have overcome the apparent deficiency induced by folacin and vitamin B_{12} was not determined.

Creatine excretion was increased from an average of approximately 1.8 mg. to an average of approximately 2.7 mg. daily per 100 g. of body weight when the basal diet was supplemented with vitamin B_{12} and folacin. Earlier, J. S. Dinning and P. L. Day (*J. Biol. Chem.* **181,** *897 (1949)*) had shown that folacin functioned in creatine formation. Addition of pyridine to the basal diet decreased creatine excretion to approximately 1.5 mg. daily per 100 g. of body weight. Vitamin B_{12} did not influence this pyridine effect but a combination of folacin and vitamin B_{12} increased creatine excretion to an average of approximately 2.3 mg. per 100 g. of body weight. This was considered as another manifestation of accelerated methylation caused by the presence in the diet of both folacin and vitamin B_{12}.

Liver choline oxidase values were also obtained by determining the oxygen consumption of buffered liver homogenates in the presence of added choline. Pyridine reduced the choline oxidase values, and this was corrected when the diet was supplemented with folacin, liver concentrate, or

vitamin B_{12}. The combination of vitamin B_{12} and folacin, however, did not increase the choline oxidase values.

None of the dietary supplements tested had any influence on blood cell, hematocrit, or hemoglobin values.

The metabolism of pyridine was investigated in 1887 by W. His (*see* R. T. Williams, "Detoxication Mechanisms," *p. 199, John Wiley & Sons, Inc., New York (1947)*) who identified the metabolic product as methylpyridinium hydroxide. Baxter (*Am. J. Path., loc. cit.*), however, was unable to obtain the methylated product in the urine of rats fed pyridine, although it could be recovered readily if the methylated product itself was fed.

The suggestion by Dinning and associates that folacin may induce a deficiency of choline in the pyridine-fed rat is an interesting one. Other investigators have observed that folacin reduces the choline requirement of the growing rat (*Nutrition Reviews* **8,** *301 (1950)*). Likewise, B. Kelley and J. R. Totter (*Fed. Proc.* **9,** *189 (1950)*) reported that folacin exerted a lipotropic effect in rats fed a diet containing 10 per cent glycine. Thus it would appear that folacin may have opposing effects on liver fat, depending upon the experimental conditions.

The suggestion by Dinning and associates that folacin may be antilipotropic under certain experimental conditions calls for more intensified studies in which liver-fat data should be obtained. Whether choline can overcome the apparent antilipotropic action of folacin should also be investigated.

EPITHELIAL PIGMENTATION AND COPPER DEFICIENCY

Achromotrichia (graying of pigmented hair) has frequently been observed in animals fed copper-deficient rations. In most such studies, this condition developed on diets composed principally of milk—a food naturally deficient in both copper and iron—and was accompanied by more or less anemia. J. M. Hundley (*Proc. Soc. Exp. Biol. Med.* **74,** *531 (1950)*), however, has observed the condition in black rats fed a purified diet, and in which the degree of deficiency was insufficient to produce either an anemia or a decrease in the birth rate.

The ration used resembled those used in vitamin studies with rats, and contained casein ("vitamin-free"), sucrose, Wesson oil, cystine, and the known vitamins required by rats; it also contained 4 per cent of the Osborne-Mendel salt mixture. This diet permitted good growth and normal hemoglobin levels; nevertheless, after eight weeks on the diet approximately 80 per cent of the animals exhibited a marked graying. This could be prevented by supplementation of the diet with any of several natural materials (e.g., liver, yeast, alfalfa) or by pure copper salts. Separate trials showed that pantothenic acid and para-aminobenzoic acid failed to influence the course of the graying, and that the ash of the effective crude supplements contained the active material supplied by these supplements.

It appeared obvious that this ration contained insufficient amounts of copper. The Osborne-Mendel salt mixture was recommended way back in 1917, before the important role played by copper in nutrition was appreciated. No copper salts were added to this mixture; however, the trace metals usually are present in synthetic rations as impurities in sufficient amounts to cover the requirements for them. Direct analysis of the diet used here showed 14.6 micrograms of copper per gram of diet, only 1 microgram of which was supplied by the salt mixture. The incidence of graying was reduced to zero by supplying an additional 12.5 micrograms of copper per gram of diet.

From these results, it appears that more copper is required to prevent achromotrichia than is required to prevent anemia. This is

in agreement with a report by F. J. Gorter (*Nature* **136,** *185 (1935)*).

A probable explanation for the occurrence of achromotrichia in copper deficiency lies in the essential role of a copper-containing enzyme, tyrosinase, in the oxidation of tyrosine to dihydroxyphenylalanine ("dopa"), and of the latter to intermediates which serve as precursors of the melanin pigment of hair and other epithelial tissues. A reduction in the amount of tyrosinase, by virtue of a reduction in copper intake, would be expected to reflect itself in reduced melanin formation.

The evidence for and against the view that tyrosinase functions also in the production of melanin pigments by the melanoblasts of human skin has been summarized by T. B. Fitzpatrick, S. W. Becker, A. B. Lerner, and H. Montgomery of the Mayo Foundation (*Science* **112,** *223 (1950)*). To learn more about this subject, these authors took advantage of the fact that following exposure to erythematous doses of ultraviolet light, the melanoblasts of the skin become enlarged and exhibit heightened activity.

Human volunteers were suitably irradiated, following which samples of skin were taken by biopsy from the irradiated site. Following a brief fixing in 10 per cent formalin, slices of the tissue were placed in a 0.005 M tyrosine solution buffered at pH 6.8 with phosphate and left for twenty-four hours at 5°C. They were then placed in a fresh lot of the same mixture for twenty-four hours at 37°C. Unirradiated skin from the same donor, and irradiated skin incubated in the same buffer lacking tyrosine, served as controls.

Microscopic examination after incubation showed unmistakably that the irradiated skin produced greatly enhanced amounts of melanin, and that this was produced only in the presence of tyrosine. Heating the slices at 100°C. for ten minutes destroyed the ability to produce melanin, showing that enzymatic oxidation of the tyrosine was involved. Furthermore, incubation of the skin slices in 0.01 M sodium diethyl-dithiocarbamate for six hours prior to incubation with tyrosine inhibited melanin formation completely. Since this chemical is a fairly specific inhibitor of copper-containing enzymes, human tyrosinase, like the tyrosinase of all other species so far examined, appears to require copper for its activity, and it appears that the graying produced by copper-deficient diets in animals may well be ascribed to a deficiency in this enzyme.

Low intakes of copper are required to produce disturbances in melanin formation, therefore it appears highly unlikely that addition of extra copper to human diets would affect pigmentation to any extent. Because of the bearing of these studies upon an understanding of the physiology of pigmentation, they nevertheless have high intrinsic interest for the nutritionist and physiologist.

VITAMIN E IN THE NUTRITION OF SWINE

Only scattered information is available concerning storage of vitamin E in animal tissues that are used for human consumption. Attempts have been made to improve the keeping qualities of meats by supplementation of the diet of the meat-producing animals with tocopherol (*Nutrition Reviews* **5,** *243 (1947)*).

L. E. Carpenter and W. O. Lundberg (*Ann. N. Y. Acad. Sci.* **52,** *269 (1949)*) reported that the fat of pigs which received supplemental tocopherol was more stable to oxidation than the fat of pigs not receiving the tocopherol supplement.

B. M. Watts, T. J. Cunha, and R. Major (*Oil & Soap* **23,** *254 (1946)*) had observed a slight increase in the stability of hog fats when the hogs were given tocopherol supplements on purified diets. Tocopherol supplements did not have this effect when the hogs

were receiving naturally-occurring feedstuffs. Protection from rancidity in the meat and fat of rabbits by feeding or injecting tocopherols was observed by J. E. Criddle and A. F. Morgan (*Fed. Proc.* **6,** *247 (1947)*).

J. W. Bratzler, J. K. Loosli, V. N. Krukovsky, and L. A. Maynard (*J. Nutrition* **42,** *59 (1950)*) reported studies that were designed to determine the extent to which the tocopherol content of the organs and tissues of swine could be influenced by dietary supplements of vitamin E and to gain further information on the role of tocopherols in lipid metabolism.

Five weanling barrows weighing from 32 to 42 pounds were used as experimental animals. The purified ration used consisted of the following percentage composition: casein 25; corn sugar 25; starch 40.3; roughage (Ruffex) 4; mineral mixture 5; liver fraction "L" 0.5; and methionine 0.2. The ration was supplemented, in milligrams per kilogram, as follows: thiamine 2.1; riboflavin 3.1; niacin 10.2; calcium pantothenate 14.8; pyridoxine 2.4; folacin 0.6; choline chloride 1.0; and carotene 1.2. In addition, 3 of the animals received a supplement of a concentrate of mixed tocopherols at levels of 2.87, 55.12, and 110.2 mg. per kilogram of body weight daily, respectively.

After the animals had been on the experimental diet for seventy-five days, they were slaughtered and various organs and tissues were analyzed for total tocopherols and also for gamma- and delta-tocopherols.

The tocopherol supplements increased significantly the content of total tocopherols of all organs and tissues that were studied. With one exception each increase in the level of tocopherol ingested brought about a significant increase in the tocopherol content of the organs and tissues. The value of 423.5 micrograms of tocopherols per gram of liver was the highest observed for any organ or tissue and it appeared in the pig receiving a tocopherol supplement of 55.12 mg. of tocopherol concentrates per kilogram of live weight daily.

The total tocopherol content of fat from various parts of the body (leaf, ruffle, jowl, back, and ham-facing) ranged from 0 to 21 micrograms per gram of tissue in the non-supplemented animals and from 162 to 284 micrograms per gram of tissue in the animal receiving the highest tocopherol supplement. These increased tocopherol storages resulting from mixed tocopherol supplementation of the diet were found to be chiefly the alpha form and in no case was the gamma- plus delta-tocopherol value greater than 5 per cent of the value for total tocopherols. Similar results, indicating primary storage of the alpha form, had been reported by M. L. Quaife, W. J. Swanson, M. Y. Dju, and P. L. Harris (*Ann. N. Y. Acad. Sci.* **52** *300 (1949)*) for the hen, the cow, and man.

In the control animals (no tocopherol supplement) no tocopherols were detected in the blood plasma although whole blood contained 80 to 90 micrograms per 100 ml. Total plasma tocopherol values of 248, 668, and 594 micrograms per 100 ml. were found in the animals receiving tocopherol supplements of 2.87, 55.12, and 110.2 mg. of mixed tocopherols per kilogram of body weight, respectively. The blood studies again emphasized that the alpha form of the vitamin was preferentially absorbed and explained the greater storage of this form of the vitamin.

Since no symptoms of vitamin E deficiency became apparent in the control animals receiving no tocopherol supplement, the results indicated that swine have a low requirement for this vitamin. F. Adamstone, J. L. Krider, and M. F. James (*Ann. N. Y. Acad. Sci.* **52,** *260 (1949)*) have reported, however, that the reproductive performance of sows kept on vitamin E-low diets was greatly lowered and that muscular incoordination appeared in their offspring.

Tocopherol supplementation of the basal diet, which contained at most only traces of fat, brought about a significant increase in the iodine and thiocyanogen values of the

body fat. The results indicated that oleic acid in the body fats was increased at the expense of the saturated fatty acids. The average oleic acid content of the fats from the control animals was 63.7 per cent as contrasted with an average value of 71.5 per cent for the animals receiving the tocopherol supplements.

The percentage of saturated fatty acids in the fat of unsupplemented animals was 35.6, contrasted with 27.3 per cent in the tocopherol-supplemented animals. The lowest level of tocopherol supplementation appeared to be as effective as the higher levels in influencing the composition of the body fat. This result suggests that vitamin E may be concerned with the synthesis of oleic acid. The tocopherol supplements did not influence the linoleic acid content of the body fats and the authors concluded that swine, like rats, are unable to synthesize this fatty acid.

Experimental evidence of the importance of vitamin E in the nutrition of farm animals has accumulated rapidly in recent years. Such evidence obviously emphasizes that this vitamin affects many tissues and apparently many biochemical systems (*Nutrition Reviews* **6,** *346* (*1948*); **8,** *135* (*1950*)). Its value in human nutrition, however, is still obscure.

RETICULOCYTE RIPENING AND AMINO ACIDS

Reticulocytes disappear progressively in blood incubated *in vitro* at body temperature (O. H. P. Pepper, *Arch. Int. Med.* **30,** *801* (*1922*)). This same process probably occurs in the circulating blood and represents the last stage in the maturation of young red cells (C. W. Heath and G. A. Daland, *Arch. Int. Med.* **46,** *533* (*1930*)). Disturbances in this ripening process have been observed in various pathologic conditions such as pernicious anemia, aplastic anemia, and chronic infections.

A. Nizet and F. S. Robscheit-Robbins (*Blood* **5,** *648* (*1950*)) studied the effect of experimental hemorrhagic anemia and of combined anemia and hypoproteinemia on the evolution of the reticulocytes of dogs. The dogs were made anemic and hypoproteinemic by methods similar to those used for many years by the Rochester group.

The "simple anemia" dogs were reduced to a hemoglobin level of 5 to 6 g. per 100 ml. by blood withdrawal and a minimum hemoglobin-producing diet. The "doubly depleted" dogs were both anemic and hypoproteinemic as a result of blood withdrawal and a nonprotein basal diet.

Reticulocytes were studied using blood stained with brilliant cresyl blue. The cells were then examined in dried smears under a dark-field microscope. Using this technic the reticulocytes were clearly distinguishable and could be differentiated into five groups according to the amount and arrangement of the reticular substance in the cells. These groups correspond to progressive stages in the ripening process. Thus group 1 represents very immature reticulocytes while group 5 is the stage just before the reticulocyte becomes an erythrocyte (A. Nizet, *Acta biol. Belg.* **2,** *170* (*1942*)). By determining the number and group distribution of reticulocytes in the blood of a given animal before and after incubation of the cells in a heparin-glucose-sodium chloride medium, ripening could be measured by a decrease in the total number of reticulocytes and by a shift toward more mature forms after incubation.

A figure approximating the time required for complete ripening of the immature reticulocyte could be derived by determining the speed with which the cells changed from less mature to more mature groups. The derivation of this figure required some extrapolation and while it might be questioned whether this ripening time is a

precise measure of the time required for the complete maturation of an immature reticulocyte, this procedure did give fairly consistent results which were useful for comparative purposes.

With but one exception, normal nonanemic dogs on kennel ration showed 12 to 38 reticulocytes per 1000 red blood cells. The time for reticulocyte ripening in these dogs varied from nineteen to forty-three hours. About one-third of the reticulocytes graduated to become mature erythrocytes after incubation periods of five and a half to eight and a half hours.

Nine simple anemia dogs on a basal salmon-bread ration showed reticulocytes in only slightly greater numbers than the nonanemic dogs. Likewise the ripening time and the percentage of shift from reticulocytes to mature erythrocytes were similar to the nonanemic dogs in incubation experiments.

Additional experiments were done using simple anemia dogs given diets of low and high potency for hemoglobin regeneration. Again the reticulocyte ripening time was similar to previous experiments and did not vary appreciably with the hemoglobin-regenerating potency of the diet.

In contrast, dogs having both anemia and hypoproteinemia showed a blockage in reticulocyte ripening. These doubly depleted dogs had about the same number and group distribution of reticulocytes as did the simple anemia dogs. However, when their cells were incubated for six to eight hours, only about 5 per cent of the reticulocytes disappeared (ripened) as contrasted to 38 per cent in the simple anemia dogs.

The ripening process was so delayed that a figure representing ripening time could not be determined. A nonprotein diet for about two to three weeks combined with frequent blood withdrawals were necessary to produce this block. Diets with varying capacities for hemoglobin regeneration were employed in doubly depleted dogs and the reticulocyte ripening studied. In general, there was a close relationship between the potency of the diet for hemoglobin regeneration and ripening time, in that a diet with good hemoglobin-regenerating properties restored the ripening time to normal.

The block of reticulocyte ripening in doubly depleted dogs could also be overcome by adding 1 per cent of a mixture of the ten essential amino acids plus glycine to the red cells during incubation. The average decrease in the total reticulocyte count during eight incubation experiments was only 6 per cent without amino acid supplements. With amino acids, the average decrease was 35 per cent. The effect was not further improved by additional nonessential amino acids.

Variable results were obtained in different dogs when one of the eleven amino acids was withheld from the incubating red cells. In some experiments with incomplete amino acid mixtures, correction of the block was obtained, either totally or partially. It seems possible that some of the amino acids may have been insufficiently depleted in some of the dogs subjected to the double depletion procedure.

Addition of the eleven amino acids to the incubating red cells of dogs with simple anemia had no influence on reticulocyte ripening. It would seem, therefore, that the block in doubly depleted dogs was due to lack of some protein factor or factors. Since addition of the eleven amino acids would correct this defect *in vitro*, it seems possible that the primary effect of the double depletion procedure was to lower the concentration of amino acids in the blood stream to a point where it was insufficient for the synthesis of hemoglobin which occurs normally in the immature red cell as it develops. Other explanations are possible, of course.

Only further experimentation can provide a firm answer to the mechanism of this effect. Nevertheless these experiments do show clearly that protein nutrition can be a factor in the maturation of immature red cells. However, it is only one of many fac-

tors which may interfere in this process. It should also be noted that a rather drastic protein depletion procedure was necessary to demonstrate this effect. Whether man is ever subjected to protein depletion of the severity necessary to make this effect of importance clinically is not clear.

All of the essential amino acids were necessary to overcome, consistently, the reticulocyte ripening block, hence it does seem possible that individuals subsisting over long periods on diets containing insufficient amounts of one or more of the essential amino acids might be subject to these abnormalities, although this must be determined by further experimentation.

WERNICKE'S SYNDROME

Clouding of consciousness and ophthalmoplegia are the chief clinical manifestations of Wernicke's syndrome. The most important pathologic change is hemorrhage in the brain. The condition is most commonly seen in chronic alcoholism and is thought to be the result of a nutritional deficiency; a deficiency of thiamine seems to be most important cause of the disease (*Nutrition Reviews* **1,** *24, 61 (1942)*; **3,** *184 (1945)*).

R. Jeremy (*Med. J. Australia* **1,** *302 (1950)*) studied a case of classic Wernicke's disease, not in an alcoholic, but in a patient suffering from severe vomiting of pregnancy which had been present for about two months prior to the first admission to the hospital.

At the time of admission intravenous dextrose and saline were given but the vomiting continued. About two weeks later the patient complained of double vision and the next day "appeared to be seeing things, particularly with her visitors, and rambled in her conversation."

Ophthalmoplegia then appeared, sluggish knee and ankle jerks were found, and a left extensor plantar response was observed. Except for obesity, the general nutritional status of the patient was good.

Consciousness was clouded, visual acuity was diminished, both pupils were dilated and failed to react to light, and there was a bilateral sixth nerve palsy. Fresh retinal hemorrhages were present. Knee and ankle jerks and abdominal reflexes disappeared and there was a left extensor plantar response. In addition there was widespread cyanotic mottling of the skin. Hyperglycemia and glycosuria, and a moderate azotemia were noted. Anemia was not present.

A diagnosis of Wernicke's encephalopathy was made, and 100 mg. of thiamine chloride were given intravenously the evening of admission. Insulin was also given. By the next morning the patient's state of consciousness had improved greatly and she was able to see considerably better. Her pupils were still dilated but reacted to light and accommodation.

Thiamine administration was continued together with the addition of niacin, ascorbic acid, and a liver extract. After seven days the ophthalmoplegia disappeared; the abnormal plantar response persisted to the twelfth day. Retinal hemorrhages gradually reabsorbed. The evidences of peripheral neuropathy were slow in clearing.

The case seems to be a classic example of Wernicke's disease and of the prompt response to administration of thiamine. This is further evidence that the disease is due, at least in large measure, to a specific deficiency of thiamine. It is possible that insulin also played a part in the cure, but this seems doubtful since only 15 units were given and the glycosuria completely disappeared following thiamine administration.

NOTES

Letter to the Editor

Dear Sir:

I was much interested in your review on the "Role of Vitamin B_{12} in the Metabolism of Methionine" (*Nutrition Reviews* **8,** *301 (1950)*). However, I would like to call to your attention certain work not cited by you which was performed in our laboratories (A. Aschkenasy and P. Pariente, *Compt. rend. Acad. d. sci.* **230,** *2339 (1950)*) and which is of some interest in the understanding of the physiologic functions of vitamin B_{12}.

In these experiments adult rats were rendered anemic by protein starvation for sixty days and then for forty days afterwards were given a synthetic diet containing only 7 per cent devitaminized casein.

Vitamin B_{12} and methionine were administered separately as supplements to the diet. Both substances accelerated the weight and blood refection. The recovery of weight was better with methionine than with vitamin B_{12}, but both increased particularly the weight of the liver and of the lymphoid organs ("Revue d'Hematologie," *in press*).

The restoration of the hematopoiesis showed some peculiarities with each of these nutrients. Thus vitamin B_{12} enhanced the reticulocyte crisis and the discharge of erythroblasts which occur in the first days of the refection of protein starvation (A. Aschkenasy and P. Aschkenasy-Lelu, *Sang* **18,** *544 (1947)*; *Arch. Sci. physiol.* **1,** *225 (1947)*). Methionine, less effective on the discharge of the immature erythrocytes, increased on the contrary, the mean corpuscular volume and suppressed the microcytosis due to the protein depletion ("Revue d'Hematologie," *loc. cit.*).

When vitamin B_{12} and methionine were given together the ponderal refection was the same as with methionine alone, but the reparation of the anemia was more important, for there was summation of the hematopoietic effects of both nutrients.

Thus vitamin B_{12} seems to contain two different fractions, one of which (probably the dimethylbenziminazolic one) is anabolic and can be substituted by methionine, the other (probably the cobalt-containing one) being only hematopoietic.

ALEXANDRE ASCHKENASY, M.D.
Paris, France

Armed Forces Rations

The Associates, Food and Container Institute, Inc., 1849 W. Pershing Road, Chicago 9, Illinois, have published an interesting pamphlet entitled "Operational Rations Used by the Armed Forces." The purpose of this booklet is to provide the essential facts pertaining to operational rations, ration supplements, and food packets used by the armed forces. In addition to a brief description and the intended purpose of each ration, such data as weight, components, and caloric values are given.

The rations described are the Operational B, Small Detachment Five-In-One, Individual Combat, and Individual Trail. Ration supplements considered are the Sundries Pack; Special Item Pack, Hospital; Special Item Pack, Aid Station; and Spice Pack, Kitchen. Food packets discussed are the Individual Assault; Individual Combat In-Flight; Lunch Air Crew; Individual Survival; Survival; and Life Raft.

Comparing these new rations with those of only a few years ago it is evident that great improvement has been made.

CLINICAL NUTRITION BRIEFS

"There was no delay in the heights and weights of British schoolchildren between 1940 and 1943. The only observed abnormality was that children living in country groups exhibited a slower rate of growth than children living with their families, despite an adequate diet. A height-weight relationship gave a better correlation with the general state of nutrition (as estimated by clinical examination) than did the standard weight curve." (*Nutrition Reviews*, Jan. 1951, p. 4, Reversibility of the Effects of Undernutrition in Children.)

"An important area of research in nutrition that promises to be of considerable practical importance is the differential influence of diet upon growth and physiologic well-being of parasite and host. Differences may be due to specific differences in requirement for dietary essentials or to unlike availability of nutrients; and the very improvement in metabolism in the host under one favorable nutritional adjustment may result in physiologic inhibition in the parasite and vice versa." (*Nutrition Reviews*, Jan. 1951, p. 8, Nutrition of Host and Parasite.)

"The proportion of erupted permanent teeth with evidence of caries experience (*i.e.*, decayed, missing, or filled teeth; abbreviated as DMF) decreased in each successive examination period in Newburgh from 21 per 100 prior to fluoridation to 14.8 per 100 at the three-year mark. By contrast, in Kingston the DMF rate remains at approximately 21 for all three years. This suggests that a 30 per cent improvement has occurred in the DMF rate in Newburgh." (*Nutrition Reviews*, Jan. 1951, p. 21, Preliminary Reports from Newburgh-Kingston.)

"Diagnosis of ariboflavinosis (riboflavin deficiency) in man is usually based on one or more of the following: evidence of a deficient food intake, visible deficiency lesions, or the blood level or urinary excretion of the vitamin. Physical manifestations, such as angular stomatitis or corneal vascularization are non-specific for riboflavin deficiency and may appear in other deficiencies. Accurate estimates of previous riboflavin intake are usually not easily obtainable in patients." (*Nutrition Reviews*, Feb. 1951, p. 49, Riboflavin Intake Versus Excretion.)

"Anemia usually accompanies the clinical syndrome of scurvy, but is variable in type. It is wholly possible that those individuals with scurvy and with manifestations of macrocytic anemia may have combined deficiencies of ascorbic acid and pteroylglutamic acid." (*Nutrition Reviews*, Feb. 1951, p. 52, Deficiencies of Ascorbic Acid and Pteroylglutamic Acid in Anemia.)

"Of the 75 patients studied in the papers covered by this review, approximately one-third had a clearcut reduction in blood pressure attributable to the rice diet, most importantly to its low sodium content. Although this dietary therapy is not a panacea for the treatment of all patients and all types of hypertensive disease, it is unquestionably an effective measure in many patients. Further investigations must attempt to make the diet more palatable and liberal so that it can be more widely applied. The mechanism of the effectiveness of the diet must be studied." (*Nutrition Reviews*, Mar. 1951, p. 70, Dietary Treatment of Hypertension. I. Clinical Studies.)

"The observation that cardiac failure is involved in vitamin E deficiency in cattle has tempted some clinicians to use this vitamin in the treatment of human cases of heart disease. Critical clinical studies have indicated, however, that vitamin E is of no value in the treatment of human heart ailments." (*Nutrition Reviews*, Mar. 1951, p. 76, Vitamin E in the Nutrition of Cattle.)